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UTILITY PATENT APPLICATION TRANSMITTAL

(Only for new nonprovisional applications under 37 C F R § 1 53 (b))

Attorney Docket No.	8070-PA01
First Inventor or Application Identifier	Jürgen Wolfrum
Title	METHOD AND DEVICE FOR QUANTIFYING DNA AND RNA
Express Mail Label No	EL584706071US

APPLICATION ELEMENTS

See MPEP chapter 600 concerning utility patent application contents

ADDRESS TO: **Assistant Commissioner for Patents**
Box Patent Application
Washington, DC 20231

1. [X] *Fee Transmittal Form *(e.g., PTO/SB/17)*
(Submit an original and a duplicate for fee processing)

2. [X] Specification [*Total Pages* 15]
(preferred arrangement set forth below)
- -Descriptive title of invention
- -Cross References to Related Applications
- -Statement Regarding Fed sponsored R & D
- -Reference to Microfiche Appendix
- -Background of the Invention
- -Brief Summary of the Invention
- -Brief Description of the Drawings *(if filed)*
- -Detailed Description
- -Claims(s)
- -Abstract of the Disclosure

3. [X] Drawing(s) *(35 U.S C. 113)* [*Total Sheets* 7]

4. Oath or Declaration [*Total Pages*]
- a. [ ] Newly executed (original or copy)
- b. [ ] Copy from a prior application (37 C.F.R. § 1.63 (d))
(for continuation/divisional with Box 16 completed)
  - i. [ ] DELETION OF INVENTOR(S)
Signed Statement attached deleting inventor(s) named in the prior application, see 37 C.F.R. §§1.63(d)(2) and 1 33(b)

NOTE FOR ITEMS 1 & 13: IN ORDER TO BE ENTITLED TO PAY SMALL ENTITY FEES, A SMALL ENTITY STATEMENT IS REQUIRED (37 C.F.R. § 1.27), EXCEPT IF ONE FILED IN A PRIOR APPLICATION IS RELIED UPON (37C.F.R. § 1.28).

5. [ ] Microfiche Computer Program (*Appendix*)

6. Nucleotide and/or Amino Acid Sequence Submission
(if applicable, all necessary)
- a [ ] Computer Readable Copy
- b. [ ] Paper Copy (identical to computer copy)
- c. [ ] Statement verifying identity of above copies

ACCOMPANYING APPLICATION PARTS

7. [ ] Assignment Papers (cover sheet & document(s))
8. [ ] 37 CFR §3 73(b) Statement *(when there is an assignee)* [X] Power of Attorney
9. [ ] English Translation Document *(if applicable)*
10. [ ] Information Disclosure Statement (IDS)/PTO-1449 [ ] Copies of IDS Citations
11 [ ] Preliminary Amendment
12. [x] Return Receipt Postcard (MPEP 503)
(Should be specifically itemized)
13. [ ] *Small Entity Statement(s) *(PTO/SB/09-12)* [ ] Statement filed in prior application, Status still proper and desired
14 [ ] Certified copy of Priority Documents(s)
(if foreign priority is claimed)
15. [X] Other Check for $345, copy of PCT WO99/50446

16. **If a CONTINUING APPLICATION,** *check appropriate box and supply the requisite information below and in a preliminary amendment*

[ ] Continuation [ ] Divisional [ ] Continuation-in-part (CIP) of prior Application No

Prior application information *Examiner* _____ *Group / Art Unit* _____

For CONTINUATION or DIVISIONAL APPS only: The entire disclosure of the prior application, from which an oath or declaration is supplied under Box 4b, is considered a part of the disclosure of the accompanying continuation or divisional application and is hereby incorporated by reference. The incorporation can only be relied upon when a portion has been inadvertently omitted from the submitted application parts.

17. CORRESPONDENCE ADDRESS

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Signature		*Date*	September 25, 2000

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See 37 C F.R. §§ 1 27 and 1 28.

TOTAL AMOUNT OF PAYMENT ($) 345.00

Complete if Known	
Application Number	Unassigned
Filing Date	Herewith
First Named Inventor	Jürgen Wolfrum
Examiner Name	Unassigned
Group / Art Unit	Unassigned
Attorney Docket No	8070-PA01

METHOD OF PAYMENT (check one)

1. ☐ The Commissioner is hereby authorized to charge indicated fees and credit any over payments to

Deposit Account Number: 02-4070

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2. ☒ **Payment Enclosed:** ☒ Check ☐ Money Order ☐ Other

FEE CALCULATION

1. BASIC FILING FEE

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
101	690	201	345	Utility filing fee	345.00
106	310	206	155	Design filing fee	
107	480	207	240	Plant filing fee	
108	690	208	345	Reissue filing fee	
114	150	214	75	Provisional filing fee	

SUBTOTAL (1) ($) 345.00

2. EXTRA CLAIM FEES

		Extra Claims		Fee from below	Fee Paid
Total Claims	16	-20**=	0	X =	0
Independent Claims	3	- 3**=	0	X =	0
Multiple Dependent				=	

***or number previously paid, if greater* *For Reissues, see below*

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description
103	18	203	9	Claims in excess of 20
102	78	202	39	Independent claims excess of 3
104	260	204	130	Multiple dependent claim, if not paid
109	78	209	39	**Reissue independent claims over original patent
110	18	210	9	**Reissue claims in excess of 20 and over original patent

SUBTOTAL (2) ($) 0

FEE CALCULATION (continued)

3. ADDITIONAL FEES

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
105	130	205	65	Surcharge - late filing fee or oath	
127	50	227	25	Surcharge - Late provisional filing fee or cover sheet	
139	130	139	130	Non-English specification	
147	2,520	147	2,520	For filing a request for reexamination	
112	920*	112	920*	Requesting publication of SIR prior to Examiner action	
113	1,840*	113	1,840*	Requesting publication of SIR after Examiner action	
115	110	215	55	Extension for reply within first month	
116	380	216	190	Extension for reply within second month	
117	870	217	435	Extension for reply within third month	
118	1,360	218	680	Extension for reply within fourth month	
128	1,850	228	925	Extension for reply within fifth month	
119	300	219	150	Notice of Appeal	
120	300	220	150	Filing a brief in support of appeal	
121	260	221	130	Request for oral hearing	
138	1,510	138	1,510	Petition to institute a public use proceeding	
140	110	240	55	Petition to revive - unavoidable	
141	1,210	241	605	Petition to revive - unintentional	
142	1,210	242	605	Utility issue fee (or reissue)	
143	430	243	215	Design issue fee	
144	580	244	290	Plant issue fee	
122	130	122	130	Petitions to the Commissioner	
123	50	123	50	Petitions related to provisional applications	
126	240	126	240	Submission of Information Disclosure Stmt	
581	40	581	40	Recording each patent assignment per property (times number of properties)	
146	690	246	345	Filing a submission after final rejection (37 CFR 1 129(a))	
149	690	249	345	For each additional invention to be examined (37 CFR 1 129(b))	
				Other fee (specify) _____	
				Other fee (specify) _____	

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SUBTOTAL (3) $ 0

SUBMITTED BY					Complete *(if applicable)*
Name (Print/Type)	James W. McClain	*Registration No. (Attorney/Agent)*	24,536	*Telephone*	(619) 238-0999
Signature				*Date*	September 25, 2000

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Method and Device to Quantify DNA and RNA

The invention concerns a device and a method to quantify DNA and RNA sequences. In particular, the invention concerns a method and a device to detect the amplification of a DNA and/or RNA sequence in a sample, especially the online detection of the amplification of a DNA and/or RNA sequence in a sample.

The detection of special DNA and RNA sequences in a sample by adding a complementary DNA or RNA sequence to the sample is a common diagnostic method. To evaluate the result, the amplification process must be observable or detectable. This is preferably done by quantifying the amplification process.

In the ABI TaqMan method, fluorescent energy transfer is used. The TaqMan probe (an oligonucleotide that hybridizes on the template on the location up to which the template is built during the PCR reaction) is marked at the 5 or 3-end of the oligonucleotide with a donor or acceptor dye. However, only the acceptor fluorescence is detectable since the donor fluorescence is quenched by the energy transfer to the acceptor. After the template has been successfully synthesized during the PCR reaction, the 5-terminal base of the TaqMan probe is digested. The close, fixed contact with the acceptor dye is lost, and the fluorescence signal rises. For reference, a free dye that emits at a different wavelength is added to the solution.

A disadvantage of this method is that adding the fluorescent dye required for measurement contaminates the sample. The sample cannot be directly processed further. The required purification steps may render the sample useless if the purification is unsuccessful.

The problem of the present invention is therefore to present a method and device to quantify DNA and RNA sequences that are easier and more efficient than the state-of-the-art methods and devices.

This problem is solved by the method and device in the independent claims. Other advantageous developments are presented in the dependent claims.

In particular, the problem is solved by a method for online detection of the amplification of a DNA and/or RNA sequence in a sample where the amplification of the DNA and/or RNA sequence in the sample is evaluated using the scattered light signal of the sample. It was surprisingly found that the amplification of DNA or RNA can be detected online without additives. The scattered light signal of the RNA or DNA molecules is used for this purpose. The method according to the invention is based on the fact that the intensity of the Rayleigh scatter (particle size $<<\lambda$, wavelength of light) is proportional to the light intensity I_0, molecule size M_c, and the concentration of the particles.

$$I \sim I_0 M_c \, c$$

By exploiting the scattered light signal, it is no longer necessary to use fluorescent dyes such as FAM, JOE, TAMRA and ROX. By continually measuring the scattered light signal, the amplification can be quantitatively determined.

In another preferred method according to the invention, the sample is excited by a source which can be a light source, preferably a lamp, laser, or light-emitting diode. A xenon lamp or a helium-neon laser is particularly preferred. In this preferred embodiment of the present invention, sources with a wide range of emission spectra can be used by obtaining information from the scattered light signal. It is therefore not necessary as in the state of the art to use a special laser light source.

In another preferred exemplary embodiment of the present invention, a method is used in which the scattered light signal corresponds to the scattered light intensity. Such a correspondence can be recorded with a detector, especially a pin diode, to quantitatively determine the amplification.

The method according to the invention has several advantages due to its simplicity. No primer or nucleotide has to be marked, and the sample can be used directly afterwards without purification

steps. The reaction can be detected online by the scattered light intensity and hence provides a more reliable, simple and cost-effective option for detecting amplification. It is also preferable to test the amplification by determining starting and end points.

In another preferred method of the present invention, the sample contains impurities, especially foreign DNA and/or RNA sequences. Added mononucleotides prevent the amplification from being followed while measuring absorption; however, it was surprisingly found that the added mononucleotides do not interfere with the measurement of scattered light. Another advantage of the present invention is hence that the method according to the invention allows detection of the amplification of a desired DNA and/or RNA sequence in a sample even when foreign DNA, RNA, and/or proteins are present since only the increase in scattered light intensity is measured. In the online detection of amplification, the viscosity (for example) and/or other properties of the solution can hence change without influencing the method according to the invention. It is particularly preferable to use the method for detection, especially online detection, when amplifying a DNA and/or RNA sequence in a contaminated sample. Self-measurements are therefore possible of DNA or RNA samples with excess foreign DNA or RNA taken from cell cultures.

In another preferred exemplary embodiment of the present invention, a method is provided whereby the products and/or educts are quantitatively measured for known initial or final concentrations of products and/or educts. In addition to a non-selective qualitative determination of whether amplification has occurred, the method according to the invention allows quantitative measurement of the products or educts. Real-time detection (online) is preferable. The initial or end concentrations of the products/educts are preferably taken into consideration for this determination.

The method according to the invention can be used for temperature cycling amplification (polymerase chain reaction PCR (RT PCR), ligase chain reaction LCR, transcription-based amplification) as well as isothermal amplification (strand displacement amplification, nucleic acid sequence based amplification NASBA, Qβ-replicase systems) and other amplification

reactions.

The problem is also solved by a device to quantify the amplification of a DNA and/or RNA sequence in a sample that has the following components: A device to excite the sample, and a detection device. The detector can detect a scattered light signal from the sample. It was surprisingly found that this device to measure scattered light can determine the amplification of the DNA and/or RNA sequence in a sample.

In another advantageous exemplary embodiment of the present invention, the excitation device is a light source, preferably a lamp, laser or especially a LED. In this preferred embodiment of the present invention, the sources can use a wide range of emission spectra since the information is obtained from the scattered light source. It is therefore not necessary to use a laser light source with a special frequency range as in the state of the art.

Another preferred embodiment is distinguished in that the detector is a photomultiplier (PMT) and/or a CCD camera and/or a diode, and especially an avalanche photodiode (APD) and/or at least one PIN diode (16). The scattered light signal can be detected with a photomultiplier and/or a CCD camera and/or a diode. It is preferable to use a combination of differently wired PIN diodes so that the special measuring situation of the individual detectors can be taken into consideration. It is accordingly possible to preferably detect scattered light signals of predetermined frequency ranges by using filters. It is also possible to detect the signals of different PIN diodes and combine the different signals to define the end signal. It is also conceivable to use an imaging device for the detector, preferably a CCD camera. A PMT and/or ADP are preferably used if small amounts of substances are to be detected since they are very sensitive.

Another preferred device of the present invention has a scanner. A scanner can be used to transmit a special scattered light signals from the sample to the detector. In this preferred exemplary embodiment of the invention, specific scattered light signals of the special sample can be transmitted to a special detector.

In another preferred device of the invention, several sample carriers, preferably microtiter plates or capillaries are used. This makes it possible to observe and preferably scan several samples in one step. This increases the efficiency of the observation and detection methods. In addition, series of samples can be observed simultaneously, and associated measurements can be processed together.

Another preferred device according to the invention has sample carriers that can be scanned with the scanner. This makes it possible to scan the sample carrier e.g. in the x-y direction. The samples in the sample carriers are preferably in a plane and are scanned and measured sequentially. It is also possible for the sample carriers to be designed to be mobile; they can be moved so that the scanner can examine one sample after the other. In addition, it is preferable for both the sample carrier and the scanner to be mobile so that the sample carriers can be exchanged and the scanner can be swung to optimally exploit the setup and loading time for the device according to the invention. The fixed sample carrier is scanned by the moving scanner, and then the scanner is moved to another field of sample carriers while the first sample carriers are processed further or exchanged.

Another preferred device according to the invention has a scanner with a preferably moving mirror that can direct a scanning beam of the scanner. This makes it possible to fix the scanner so that it and the sample carriers do not have to be moved. In this preferred exemplary embodiment, only the mirror is moved to transfer to the detector the corresponding scattered light signals of the individual samples in the sample carriers. It is preferable for the sample carriers to be scanned (especially cyclically) in a set sequence to more-or-less continuously detect the scattered light signal of each sample using the known position of the mirror. For example, at time t1, a sample carrier P1 can be detected, at time t1 + T sample carrier P2 can be detected, etc. up to time t1 + NT where sample carrier P1 is redetected (N is the number of sample carriers P to be detected, and T is the time to measure and detect the following sample). For special sample x in sample carrier Px, the detection of the scattered light signals and hence the process of amplifying a DNA and/or RNA sequence in sample x is hence more-or-less continuous by interpolating the

measured values of sample x at times:

$$tx, tx + NT, tx + 2NT, \ldots tx + iNT, \text{etc.}$$

Another preferred embodiment of the present invention concerns a device where the excitation mechanism is designed so that the sample carriers or samples can be excited over a wide area, and the scattered light signals corresponding to the individual sample carriers can be individually detected by the detector. This makes it possible to simultaneously detect and evaluate a large-area sample or sample fields or sample carriers distributed over a large area. Continuous, diffuse excitation of the sample field is sufficient since the relative scattered light intensity that is independent of the absolute scattered light signal at each site is detected independently for each sample or section of the sample field.

In another preferred embodiment of the present invention, the device has a detecting device with at least two individual detectors that detect different scattered light signals. This makes it possible for several samples to be detected simultaneously and not sequentially. This is particularly advantageous when a precise simultaneous evaluation of the samples is desirable. It is particularly preferable to connect the individual detectors via optical fibers to the sample carriers or samples so that the profiles of the sample fields can also be detected using an optical fiber bundle. It is also preferable to record a large-area sample field with a CCD camera and detect the scattered light signals at the individual sites by evaluating the picture, preferably with a controller and especially preferably via a computer or image processing system.

It is also preferable to provide a controller to which signals are sent that correspond to the detected scattered light signals, and the controller evaluates the signals. This controller can process the individual measured values in the corresponding matrices for the individual samples and send them to a memory. In addition, a scanner can also be controlled by the controller, and the detectors can be set e.g. in regard to their sensitivity and alignment toward the samples.

In an additional advantageous use of the present invention, the device to measure scattered light

can quantify the amplification of a DNA and/or RNA sequence in a sample.

In the following, other advantageous embodiments of the invention will be explained with reference to the drawing. Shown are:

Fig. 1 A graph of a scattered light intensity measurement according to an exemplary embodiment of a method according to the invention at a given concentration (a), and a graph of a scattered light intensity measurement according to an exemplary embodiment of the method according to the invention from (a) at two diluted concentrations and a negative control (b);

Fig. 2 A graph that compares the measurement of fluorescence using the state-of-the-art TaqMan method and the measurement of scattered light (a) according to the invention, and a graph that compares the measurement of fluorescence using state-of-the-art intercalation dye and the measurement of scattered light (a) according to the invention (b);

Fig. 3 A schematic design of an exemplary embodiment of the device according to the invention with a sample;

Fig. 4 A schematic design of an exemplary embodiment of the device according to the invention with numerous samples or sample carriers and a scanner; and

Fig. 5 A schematic design of an exemplary embodiment of the device according to the invention with numerous samples or sample carriers and optical fibers.

Fig. 1a shows a graph of a scattered light intensity measurement of an exemplary embodiment of a method according to the invention at a given concentration. One can clearly see the rise in the relative scattered light intensity over time. This curve represents the advancing amplification in the sample.

Fig. 1b is a graph of a scattered light intensity measurement according to the exemplary embodiment of a method according to the invention from Fig. 1 at two diluted concentrations (curves A and B). In addition, the curve of a negative control (curve C) is also shown. This illustrates that one can also evaluate the quality by measuring the relative scattered light intensity. In the case of the negative control (curve C), there is no rise in the relative scattered light intensity. At the start of measurement, enzymes were added for A and B that triggered the amplification. No mononucleotide triphosphate was added to the negative control.

Both Fig. 1a and 1b concern AmpliScribe™ SP6 reactions (Epicentre Technologies) – a commercially available amplification of RNA by transcription – at a reaction temperature of 39°C.

Components	**Volume**	**Final Concentration**		
	Curve A/B/C	Curve A	Curve B	Curve C
ATP (100 mM)	2/2/0 µl	2.8 mM	2.3 mM	0 mM
CTP (100 mM)	2/2/0 µl	2.8 mM	2.3 mM	0 mM
GTP (100 mM)	2/2/0 µl	2.8 mM	2.3 mM	0 mM
UTP (100 mM)	2/2/0 µl	2.8 mM	2.3 mM	0 mM
DTT (100 mM)	4 µl	5.6 mM	4.6 mM	5 mM
AmpliScribe SP6 enzyme solution	4 µl			
Water	47/63/63 µl			
10x SP6 reaction buffer	7 µl	1x	0.8x	0.9x
DNA control template (0.5 µg/µl)	1 µl	1 µg	1 µg	1 µg
Total volume	**72 / 88 / 89 µl**			

Fig. 2a shows a graph that that compares the measurement of fluorescence according to the state-of-the-art and the measurement of scattered light according to the invention. The measuring points of the scattered light measurement are shown as black dots while the fluorescence measurements are shown as circles. One can easily see that amplification can be demonstrated by both measurements.

Below are the test conditions for measuring scattered light according to the invention:

Components	**Vol./reaction**	**Final conc./reaction**
ATP (10 mM)	1 µl	200 µM
CTP (10 mM)	1 µl	200 µM
GTP (10 mM)	1 µl	200 µM
TP (10 mM)	1 µl	200 µM
Primer A	Variable	0.1 µM
Primer B	Variable	0.1 µM
AqDNA polymerase (5U/µl)	0.5 µl	2.5 U

Components	**Vol./reaction**	**Final conc./reaction**
Water	Variable	
10x PCR buffer	5 µl	1x
DNA template	Variable	Ca 0.25 µg/reaction
Total volume	**50 µl**	

Cycling conditions:	95°C	120 s	
	95°C	20 s	
	60°C	30 s	
	72°C	60 s	40x cycles

After five cycles in each case, a sample is removed and diluted at the end of the 72°C step for the scattered light measurement and diluted with 50 µl water.

Fig. 2b shows a graph that compares the measurement of a fluorescence using state-of-the-art intercalation dye and a measurement of scattered light according to the invention. This comparative measurement was done with intercalating agent PicoGreen:

The same samples were used here that were used to measure scattered light intensity in Fig. 2a but only with 2 µl of the reaction solution diluted with 60 µl water and 20 µl PicoGreen (1:20 dilution) solution. The samples were excited at 480 nm, and the amplification was detected at 525 nm. One can clearly see that the success of the PCR reaction has been demonstrated in this case as well.

Fig. 3 shows a schematic diagram of an exemplary embodiment of the device according to the

invention with a sample. Sample 1 is excited by a light source 2. The light emitted by the source is guided by monochromators 18 and focused by a lens 21 on the sample. The scattered light is transferred by lens 21' via monochromator 18' to PIN diode 16 that is used as a detector. A controller 17 that evaluates and records the signals is connected to the detector.

Fig. 4 shows a schematic diagram of an exemplary embodiment of the device according to the invention with numerous samples 1 or sample carriers 15 and a scanner. Sample carriers 15 are arranged in a sample field 1. An exciter (excitation laser in this case) emits light and excites a sample Px via a lens or microscope objective 21. The scattered light is transferred via lens 21 and glass pane 4 to the detector 13. The detector is connected to a controller 17. This controller controls a scanner 14 that can move the sample field 1.

The controller sends a control pulse to the scanner and causes it to move a special sample into the focus of the lens 21. Then a measurement is made, the measured value is detected and saved, and then the scanner is controlled by another control signal from the controller that moves the next sample into the focus of the lens 21, and the sample is measured. The samples can accordingly be cyclically recorded and measured preferably in a cyclical manner.

Fig. 5 shows a schematic diagram of an exemplary embodiment of the device according to the invention with numerous samples or sample carriers and optical fibers. The detector 13 is connected via optical fibers 22 to the sample 1. The excitation light is transmitted via the optical fibers, and the scattered light is also detected via the optical fibers. The detector sends the detected signals to the controller (17) where they are processed and buffered as needed.

A device and method have been presented for the detection (especially the online detection) of the amplification of a DNA and/or RNA sequence in a sample. The amplification of the DNA and/or RNA sequence in the sample is based on the scattered light signal of the sample. This method and device to quantify DNA and RNA sequences are easier and more efficient to use online than state-of-the-art methods and devices.

Reference Number List

1	Sample
2	Source, especially a light source
12	Excitation device
13	Detector
14	Scanner
15	Sample carrier
16	PIN diode
17	Controller
18	Monochromator
19	Mirror
20	Glass plate
21	Lens
22	Optical fibers

Patent Claims

1. A method for the detection, especially online detection, of the amplification of a DNA and/or RNA sequence in a sample (1),

characterized in that

the DNA and/or RNA sequence in the sample (1) is evaluated based on the scattered light signal of the sample (1).

2. The method according to claim 1, characterized in that the sample (1) is excited by a source (2), whereby the source (2) is a light source, especially a lamp, laser or LED.

3. The method according to claim 1, characterized in that the scattered light signal corresponds to the scattered light intensity.

4. The method according to claim 1, characterized in that the sample contains impurities, especially foreign DNA and/or RNA sequences.

5. The method according to claim 1, characterized in that the quantities of products and/or educts are determined for known initial or end concentrations of products and/or educts.

6. A device comprising
means (12) for exciting a sample (1),
means for quantifying an amplification of a DNA and/or RNA sequence in the sample (1) according to the method according to claim 1 that comprises a detector (13), which can detect a scattered light signal from the sample (1).

7. The device according to claim 6, characterized in that the excitation device (12) is a light source, especially a lamp, laser or LED.

8. The device according to claim 6, characterized in that the detector (13) is a photomultiplier and/or a CCD camera and/or a diode, especially an avalanche photodiode and/or at least one PIN diode (16).

9. The device according to claim 6, characterized in that a scanner (14) is also provided.

10. The device according to claim 6, characterized in that a plurality of sample carriers (15) is provided, the sample carriers being especially selected from a group consisting of microtiter plates and capillaries.

11. The device according to claim 10, characterized in that the sample carriers (15) can be scanned with the scanner (14).

12. The device according to one of claims 9, characterized in that the scanner (14) comprises a mirror (19) that preferably moves and can be used to direct a scanning beam from the scanner (14).

13. The device according to claim 10, characterized in that
the means (12) for exciting the sample (1) is designed so that large numbers of sample carriers (15) can be excited and in that
the detector (13) is designed so that scattered light signals that correspond to individual sample carriers (15) can be individually detected by the detector (13).

14. The device according to claim 6, characterized in that the detection device (13) has at least two individual detectors that can detect different scattered light signals.

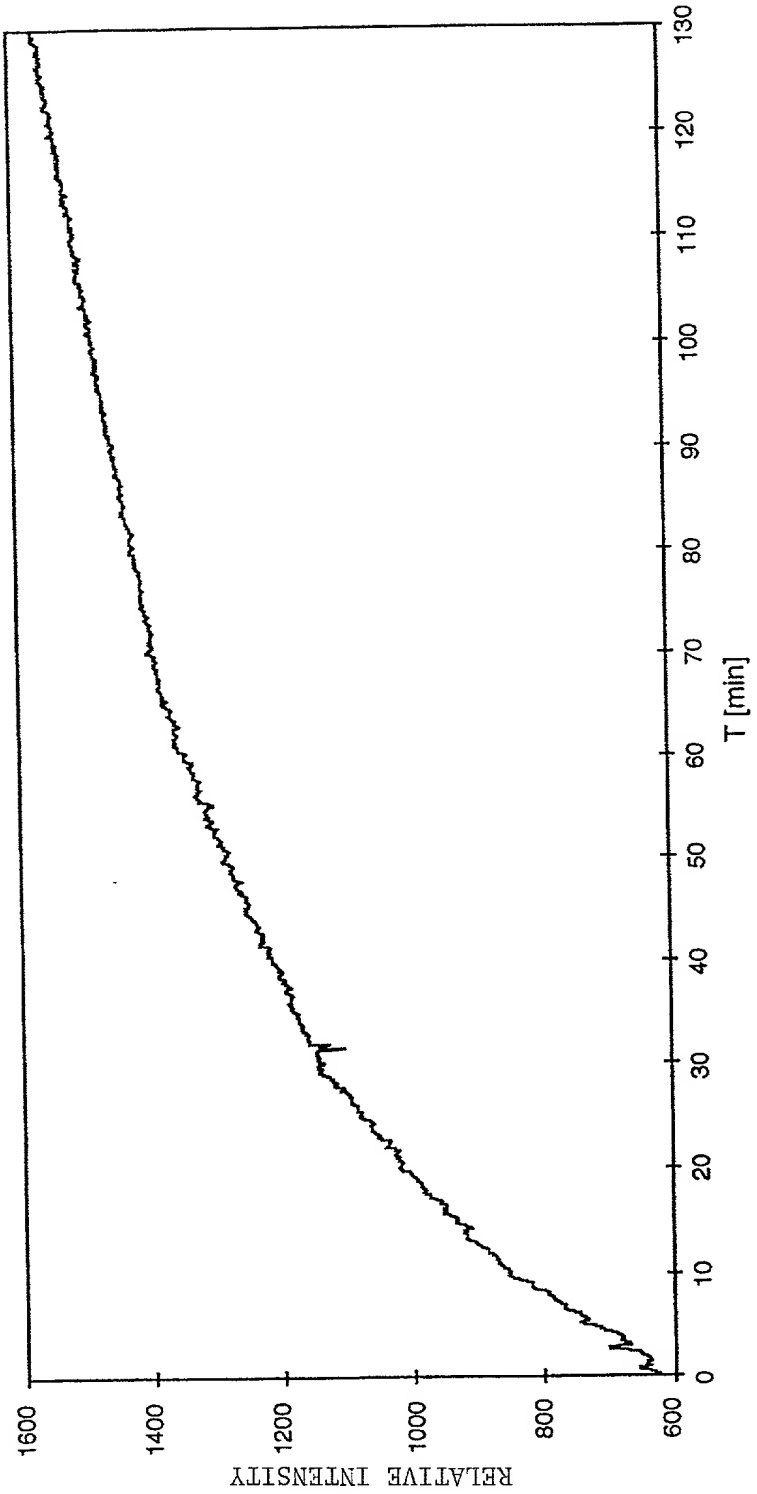
15. The device according to claim 6, characterized in that a controller (17) is also provided that can be sent and can evaluate signals which correspond to the detected scattered light signals.

16. The use of a device to measure scattered light to quantify the amplification of a DNA and/or

RNA sequence in a sample (1) according to the method according to claim 1.

ABSTRACT

The invention relates to a device and a method for the detection, especially on line detection, of an amplification of a DNA and/or RNA sequence in a sample. The amplification of the DNA and/or RNA sequence in the sample is evaluated on the basis of scattered-light signal of the sample.


RELATIVE INTENSITY
1600
1400
1200
1000
800
600
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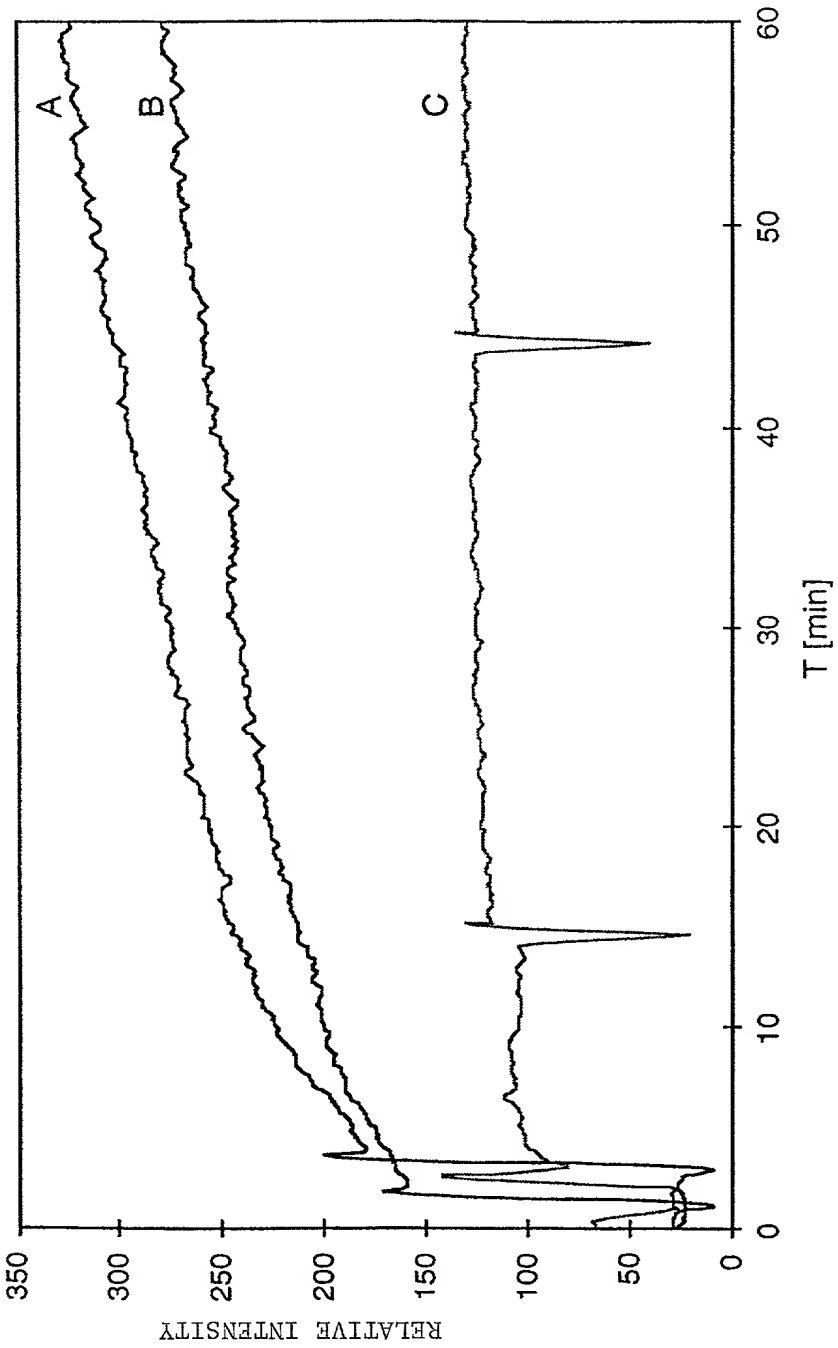
10
20
30
40
50
60
70
80
90
100
110
120
130
T [min]

Fig. 1a


A
B
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RELATIVE INTENSITY
350
300
250
200
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50
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T [min]

Fig. 1b

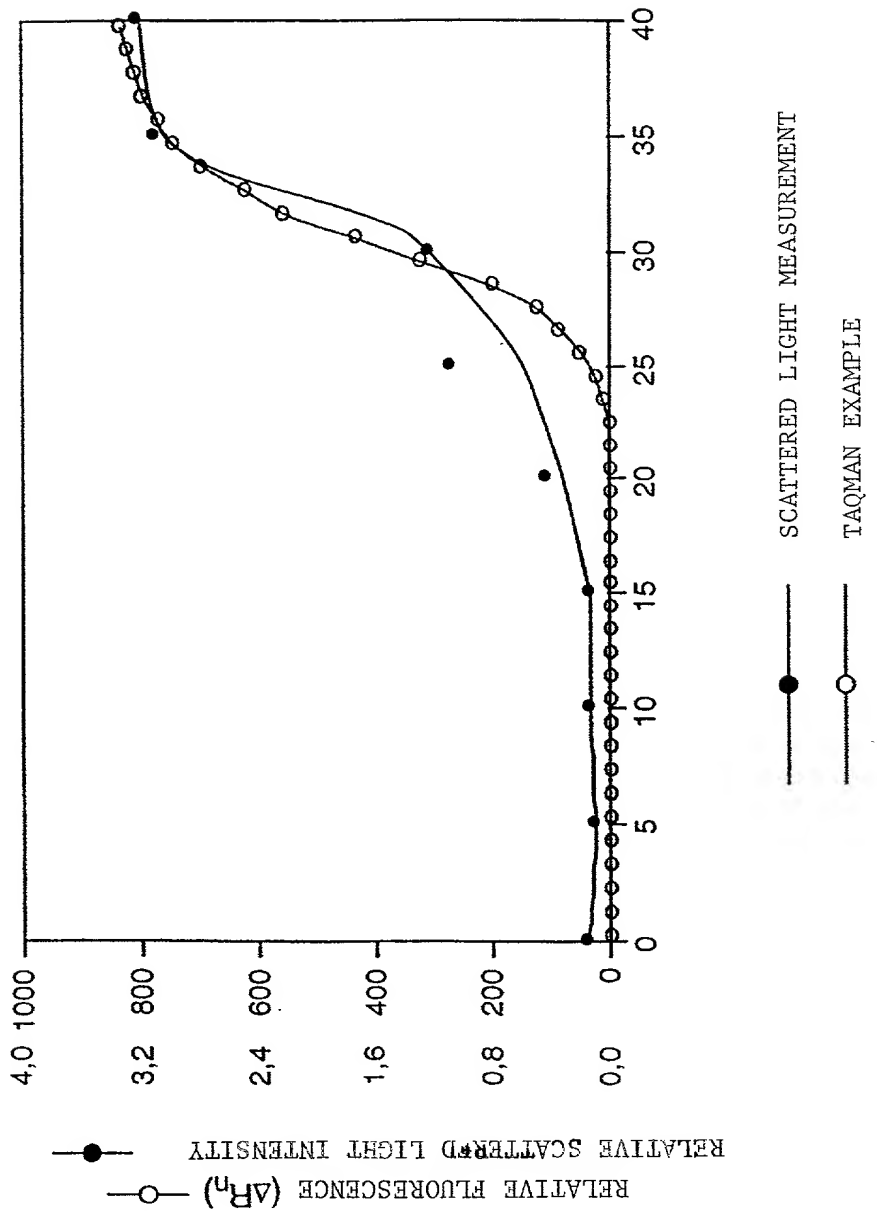
RELATIVE FLUORESCENCE (ΔR_n)
RELATIVE SCATTERED LIGHT INTENSITY
4,0 1000
3,2 800
2,4 600
1,6 400
0,8 200
0,0 0
0 5 10 15 20 25 30 35 40
SCATTERED LIGHT MEASUREMENT
TAQMAN EXAMPLE

Fig. 2a

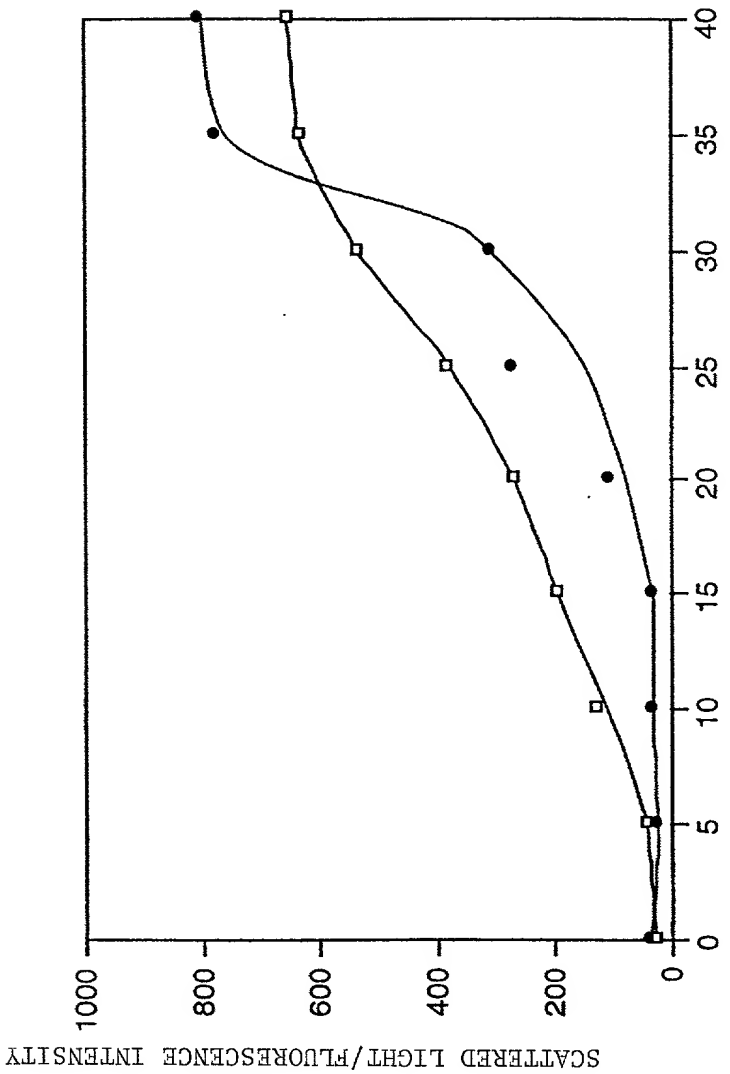
SCATTERED LIGHT/FLUORESCENCE INTENSITY
0
200
400
600
800
1000
0
5
10
15
20
25
30
35
40
SCATTERED LIGHT
FLUORESCENT MEASUREMENT WITH PICO GREEN

FIG 2B

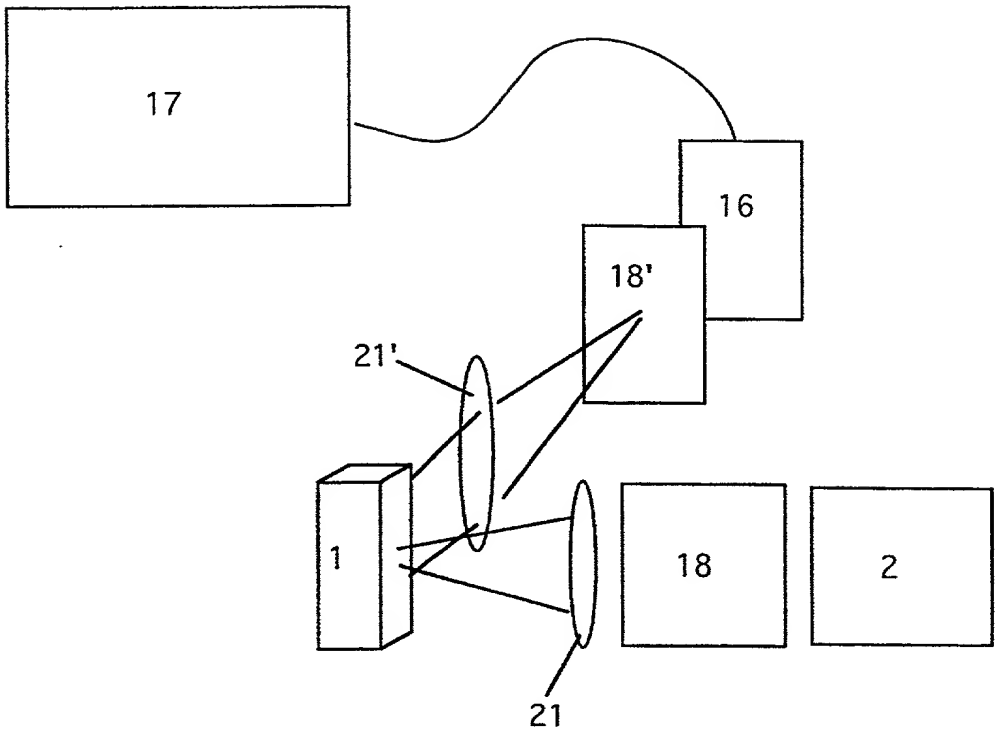

Fig. 3

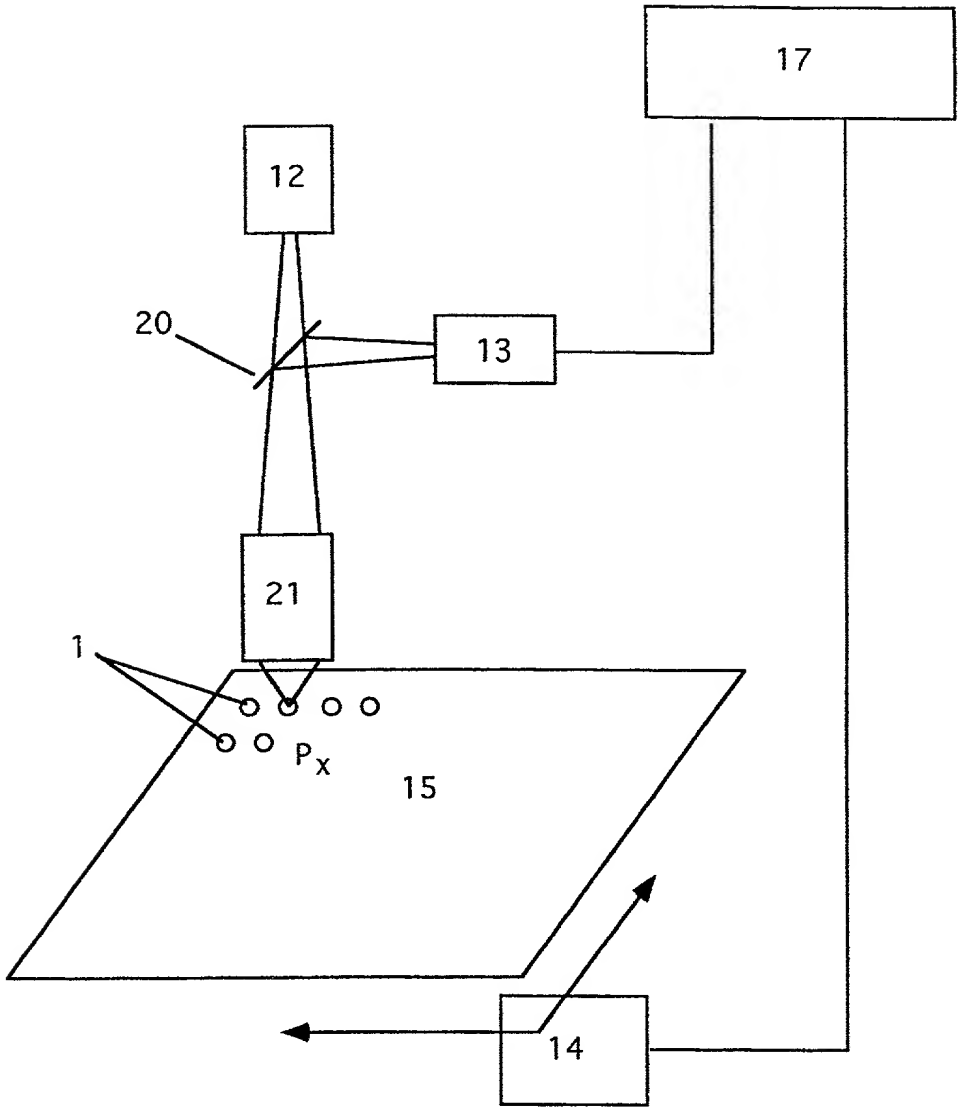

Fig. 4

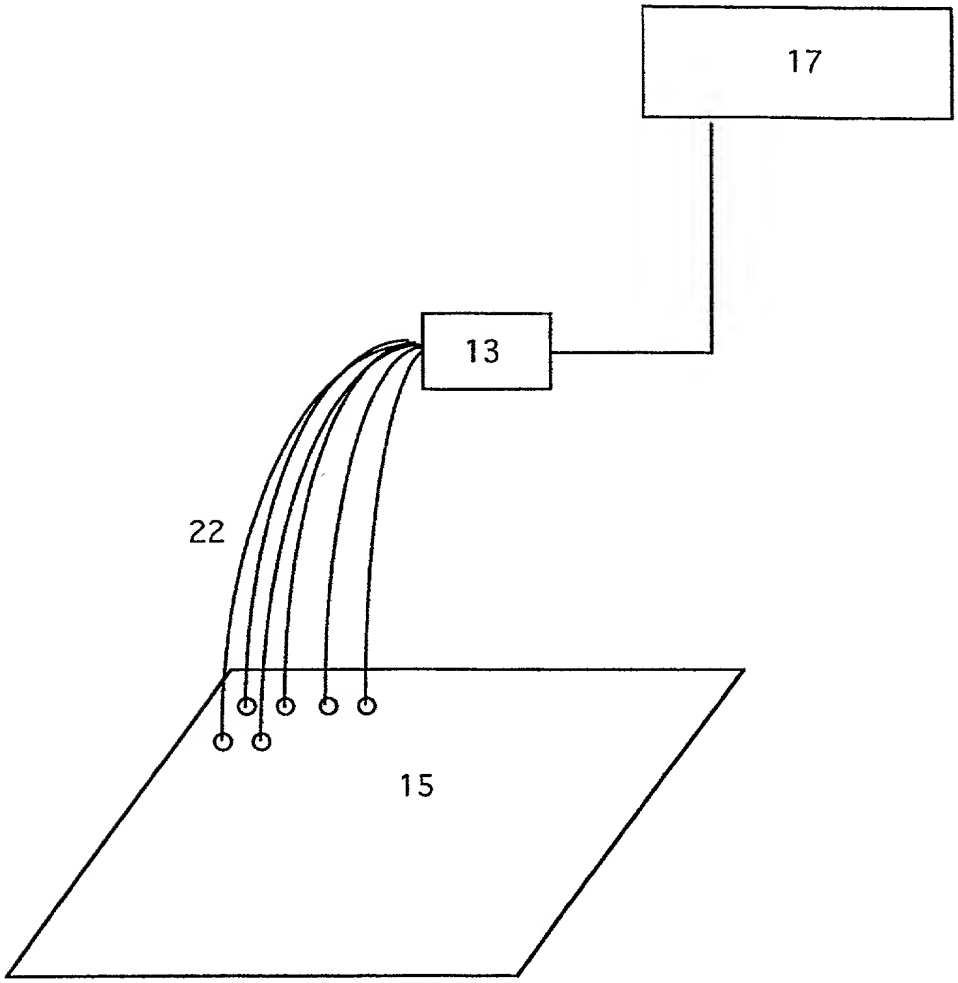

Fig. 5

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DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION

[X] Declaration Submitted with Initial Filing [ ] Declaration Submitted after Initial Filing

Attorney Docket	8070-PA01
First Named Inventor	Jürgen Wolfrum
COMPLETE IF KNOWN	
Application Number	UNKNOWN
Filing Date	HEREWITH
Group Art Unit	UNKNOWN
Examiner Name	UNKNOWN

As a below named Inventor, I hereby declare that:

My residence, post office address, and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

PROCESS AND APPARATUS FOR PREDICTION OF HUMAN GLUCOSE LEVELS

(*Title of the Invention*)

the specification of which

[X] is attached hereto

OR

[ ] was filed on (MM/DD/YYYY) [ ] as United States Application Number or PCT International Application Number [ ] and was amended on (MM/DD/YYYY) [ ] (if applicable)

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment specifically referred to above

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37 Code of Federal Regulations, §1 56

I hereby claim foreign priority benefits under Title 35, United States Code §119(a)-(d) or §365(b) of any foreign application(s) for patent or inventor's certificate, or §365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or of any PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application Numbers	Country	Foreign Filing Date (MM/DD/YYYY)	Priority Not Claimed	Certified Copy Attached? YES	Certified Copy Attached? NO
PCT/EP99/02242	PCT	04/01/1999	[ ]	[ ]	[X]
DE19814682 5	Germany	04/01/1998	[ ]	[ ]	[X]
			[ ]	[ ]	[ ]
			[ ]	[ ]	[ ]

[ ] Additional foreign application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto

I hereby claim the benefit under Title 35, United States Code § 119(e) of any United States provisional application(s) listed below

Application Number(s)	Filing Date (MM/DD/YYYY)

[ ] Additional provisional application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto

[DECEGOUG00.C01]

DECLARATION - Utility or Design Patent Application

I hereby claim the benefit under Title 35, United States Code §120 of any United States application(s), or §365(c) of any PCT International application designating the United States of America, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior Unite d States or PCT International application in the manner provided by the first paragraph of Title 35, United States Code §112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations §1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

U.S. Patent Application Number	PCT Parent Number	Parent Filing Date (MM/DD/YYYY)	Parent Patent Number (if applicable)

☐ Additional U.S. or PCT international application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto

As a named inventor, I hereby appoint the following registered practitioner(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith. Registered practitioner(s) name/registration number listed below.

Name	Registration Number	Name	Registration Number
NEIL F. MARTIN JOHN L. HALLER JAMES W. MCCLAIN	23,088 27,795 24,536		

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

NAME OF SOLE OR FIRST INVENTOR: ☐ A petition has been filed for this unsigned inventor

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☐ Additional Inventors are being named on the supplemental Additional Inventor(s) sheet(s) PTO/SB/02A attached hereto

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DECLARATION

ADDITIONAL INVENTOR(S)
Supplemental Sheet
Page 3 of 3

Name of Additional Joint Inventor, if any:	☐ A petition has been filed for this unsigned inventor						
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Name of Additional Joint Inventor, if any:	☐ A petition has been filed for this unsigned inventor						
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Post Office Address							
Post Office Address							
City		State		Zip		Country	

Name of Additional Joint Inventor, if any:	☐ A petition has been filed for this unsigned inventor						
Given Name (first and middle [if any])				Family Name or Surname			
Inventor's Signature					Date		
Residence: City		State		Country		Citizenship	
Post Office Address							
Post Office Address							
City		State		Zip		Country	